Yeast cell wall product enhanced intestinal IgA response and changed cecum microflora species after oral vaccination in chickens

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ABSTRACT The study was designed to explore the effect of a commercial yeast cell wall product (**YP**) on chicken intestinal IgA response and cecum microbiome after oral vaccination. Chickens were fed with YP during the experiments and orally immunized with live Newcastle disease virus (**NDV**) vaccine at 2 wk of age. Then, the animals were sacrificed, and samples were collected to measure the indicators of hemagglutination inhibition (**HI**), IgA response, IgA + cells, and cecum microbiome populations. The results showed that supplement of YP significantly enhanced serum NDV HI titer, intestinal NDV-specific secretory IgA, and intestinal IgA + cells. The sequencing results revealed that obviously increased relative abundance of Ruminococcaceae and decreased population of Bacteroidaceae in cecum were found in YP group. In summary, YP supplementation in diet enhanced intestinal IgA response to NDV vaccination by oral route and modulated the cecum microbiota to the advantage of the host in chickens.

Key words: yeast cell wall product, intestinal IgA response, cecum microflora, Newcastle disease virus vaccine

INTRODUCTION

The Ministry of Agriculture and Rural Affairs of China has banned all of in-feed use of antibiotics from 2020, thus has stimulated researchers and feed manufacturers to look for safer alternatives. A variety of microbial species such as *Bacillus*, *Lactococcus*, and *Saccharomyces* yeast are extensively used as probiotics in poultry production (Patterson and Burkholder, 2003; Diaz-Sanchez et al., 2015). Extracts derived from yeast cell wall has been demonstrated effectively to increase growth performance and immune responses in chickens (Sauerwein et al., 2007; Xue et al., 2017). The previous studies reported that supplement of yeast cell wall product (**YP**) significantly enhanced specific antibody levels against Newcastle disease virus (**NDV**) in broilers (Muthusamy et al., 2011). In addition, YP could improve mRNA expression of IFN-γ in broiler cecum tonsils after coccidial infection (Shanmugasundaram et al., 2013). It is presumed that the benefit from traditional oral administration of YP devoted immunomodulatory roles via the enteric mucosal immune responses.

The intestinal mucosal immune system consists of innate and adaptive defense mechanisms, which prevents microbial infections and maintains intestinal homeostasis (Luo et al., 2013). The immune responses to antigens in intestine mainly depend on various immunocompetent cells including B cells and T cells (Lee et al., 2018). IgG, IgM, and IgA secreted by B cells have multiple function mediating humoral effector mechanisms in avian species (Jeurissen et al., 2000; Chhabra et al., 2015). While IgG plays a vital role in preventing disease in peripheral blood, secretory IgA (**sIgA**) from B cells in the intestinal laminal propria serves as dominant immunoglobulin to protect mucosal surfaces (Chou et al., 2016). Gut microbes shape mucosa immune function by regulating the secretion of sIgA and prevention infections of pathogens (Kogut et al., 2020). The importance of gut microbes has been well elucidated in terms of host nutrition, development, and immunity in mammals as well as in chickens (Kohl, 2012; Shanmugasundaram et al., 2013; Alizadeh et al., 2016). However, few studies have addressed the effect of YP on intestinal mucosal immune responses and gut microbiota in chickens after oral immunization.

The present study was designed to evaluate the effect of supplementation with YP on humeral and intestinal mucosal immune responses to NDV vaccine by analyzing serum HI titers, intestinal IgA production, and number of IgA + cells. Furthermore, the effect of YP on the cecum microbial flora was also investigated using an Illumina NovaSeq platform.

MATERIALS AND METHODS

Chicken

One-day-old White Roman chickens (male) were purchased from Chengdu Muxing Poultry Co., Ltd. (Chengdu, China) and separately housed into wire cages. The room was kept at 37°C at the beginning of the pretrial period and then gradually reduced to 26°C. Feed and water were supplied ad libitum. All procedures related to the birds and their care were approved by the Southwest University Committee on Animal Care and Use.

Vaccine

Live Newcastle disease virus vaccine (Strain La Sota) was purchased from Qingdao YEBIO Bio-engineering Co., Ltd. (Qingdao, China).

Reagents

Yeast cell wall product was a product (AngelPW220) derived from yeast cell (*Saccharomyces cerevisiae*) walls containing β-glucan (≥30%) and mannan-oligosaccharides (**MOS**) (≥20%) (Angel Yeast, Yichang, China). The antigen and positive control sera used for the NDV-specific hemagglutination inhibition (**HI**) titers were obtained from Qingdao Regen Diagnostics Development Center (Qingdao, China). The Newcastle disease virus antibody test kit was a product of IDEXX Laboratories Inc. (Westbrook, Maine). Goat anti-chicken IgA antibody and anti-chicken IgA-HRP antibody was from Bethyl (Montgomery, TX). Mouse anti-goat IgG antibody was from Beyotime Bio-engineering Co., Ltd. (Shanghai, China). All other chemicals were analytic grade.

Experimental Design

In experiment 1, 75 chickens were randomly divided into 5 groups (Table 1) and assigned to the basal diet (Table 2) or the basal diet supplemented with 0.05% YP (β-glucan, 0.15 g/kg; MOS, 0.1 g/kg), 0.1% YP (β-glucan, 0.3 g/kg; MOS, 0.2 g/kg) or 0.2% YP (β-glucan, 0.6 g/kg; MOS, 0.4 g/kg) during the experiment. Then group A was vaccinated with saline, and groups B–E were orally immunized with live Newcastle disease virus vaccine at 2 wk of age. Blood samples were collected using wing vein puncture from 15 chickens of each group 1, 2 wk postimmunization for determination HI titers.

In experiment 2, 45 chickens were randomly assigned to 3 groups of 15 individuals each (Table 3). Chickens were fed 0 or 0.1% YP (β-glucan, 0.3 g/kg; MOS, 0.2 g/kg) during the experiment and orally immunized with live Newcastle disease virus vaccine or the same volume of saline at 14 d of age. Blood samples and feces were collected from all chickens before and 1, 2, 3, 4 wk postimmunization for determination of NDV-specific HI titers or NDV-specific IgA levels. Chickens were sacrificed at 4 wk postimmunization, and duodenum tissues were fixed in 10% neutral buffered formalin for routine histology. Then the cecum contents were immediately transferred to a sterile 2-mL centrifuge tube and kept in a −20°C freezer until DNA extraction.

HI Test

Serum NDV-specific HI titers were detected as previously described (Zhai et al., 2011). In brief, serum was diluted with phosphate buffered saline (**PBS**) from 1:2 to 1:2,048 in the V-shaped bottom 96-well microtiter plate. Next, 25 μL of NDV dilution was added per well and incubated at 37°C for 40 min. The last row was only added with PBS and served as the negative control. After that, a 25-μL of 1% chicken erythrocyte suspension was added to each well and incubated at 37°C for 15 min. All samples were tested in triplicate, and positive and negative controls were included on each plate. The HI titer was defined as the reciprocal titer at which no agglutination was observed. The data were expressed as log 2 of the highest dilution that exhibited complete inhibition of hemagglutination. The experiment was repeated 2 times and gave similar results.

NDV-Specific IgA in Feces

The fecal samples were mixed with washing buffer and then centrifuged at 2,000 rpm for 10 min. After that, the supernatants were collected for evaluating NDV-specific IgA levels (Beirao et al., 2018). The ELISA plates were purchased from IDEXX Laboratories Inc. and recovered to room temperature before testing. Next, 2-fold dilutions of feces samples from chickens were dispensed in the plates and incubated for 2 h at room temperature. Then, 1:10,000 dilution of HRP-conjugated goat anti-chicken IgA antibodies (Bethyl Laboratories) were applied to the wells after washing 3 times with PBS and incubated for 1 h in dark at room temperature. After washing, 100 μL of 3, 3′, 5, 5′-tetramethylbenzidine substrate solution was added to each well and incubated for

Table 1. Experimental design (experiment 1).

Group	n	YP	Vaccination
A	15	0	
B	15	0	+
C	15	0.05%	+
D	15	0.1%	+
E	15	0.2%	+

Abbreviation: YP, yeast cell wall product.

Table 2. Composition of the basal diet.[1]

Ingredient	%
Crude Protein	16.0
Crude Fiber	7.0
Ca	0.8–1.5
Total phosphorus	0.5
Digestible Lysine	0.3
Sodium chloride	0.3–0.8
H_2O	13.0

[1]Value were calculated from data provided by Feed Database in China (2013).

15 min. Finally, the reaction was stopped by adding 2 mol sulfuric acid and the optical density values were read at 450 nm with a microplate reader (iMark16851, Bio-Rad Laboratories, Hercules, CA). The experiment was repeated 2 times and gave similar results.

Immunohistochemical Staining for Mucosal IgA + Cells

The duodenum tissues were collected and sectioned in a conventional way (Yu et al., 2015). First, the sections were removed as paraffin by using xylene and rinsed with PBS 3 times, each time for 5 min. Next, the sections were placed in citrate buffer and bathed at 98°C for 20 min. When cooled to 37°C, rinse 3 times with PBS for 5 min. After 3% H_2O_2–methanol solution and 5% fetal bovine serum were added and incubated at 37°C for 10 min and 30 min, respectively, the sections were incubated with the diluted goat anti-chicken IgA (1:600, Bethyl Laboratories) at 4°C for 12 h. Then 1:1,000 dilution of HRP-conjugated rabbit anti-goat IgG antibodies (Beyotime Biotechnology, Shanghai, China) were added and incubated for 1 h at room temperature before washing. The reaction was terminated with distilled water after the 3,3′- diaminobenzidine (Boster, Wuhan, China) coloration solution was applied for 3 min. Hematoxylin was restained with 40 s, and 1% alcohol hydrochloride was differentiated with 20 s. Finally, sections were sealed by conventional methods. Sections were observed by a light microscope (Nikon Eclipse 80i, Nikon, Tokyo, Japan), and the number of IgA + cells was counted. Ten visual fields were selected from each section for statistical analysis.

DNA Extraction From Cecum Contents

A total of 12 birds (6 birds per group) were randomly chosen from group B and C in experiment 2 (Table 3) for collection of cecum content. CONTROL or group B: normal feed and immunized with NDV vaccine, YP or group C: normal feed with 0.1% YP and immunized

with NDV vaccine. Extraction of genome DNA, library preparation, sequencing, and data analysis were provided by Novogene Bioinformatics Technology Co., Ltd. (Beijing, China). Total genome DNA from each sample was extracted using the CTAB/SDS method and diluted to 1 ng/µL (Chao et al., 2019).

Library Preparation and Sequencing

The library targeting the V3 region of 16S rRNA was constructed by using DNA samples separated from the cecum contents. In brief, individual DNA samples were amplified by PCR using a specific primer with Barcode and Phusion High-Fidelity PCR Master Mix with GC Buffer (New England Biolabs, Beverly, MA). Then PCR amplicon was detected by electrophoresis using 2% agarose gel. The gel extraction kit (Qiagen, GmbH, Germany) was used to isolate the purpose gene fragments. And then, both library concentration and an exact product size were measured using TruSeq DNA PCR-Free Sample Preparation Kit through a quantitative PCR and Qubit. The PCR-free library was constructed based on the illumina Nova sequencing platform. NovaSeq6000 was used for on-board sequencing following qualified the library, and 140 bp paired-end reads were produced, and the raw-sequence data of the whole experiment have been submitted to https://submit.ncbi.nlm.nih.gov/subs/bioproject/SUB8069709/overview.

Statistical Analysis

One-way analysis of variance with Duncan post hoc test was employed for multiple comparisons between groups using SPSS software (version 20.0, SPSS Inc., Chicago, IL). Data were expressed as the mean ± SE. $P < 0.05$ or $P < 0.01$ were considered statistically significant.

The raw data from 16S rDNA amplicon sequencing is filtered through QIIME (Version 1.9.1) quality control process to obtain high-quality tags. The final good tags were clustered into OTU at 97% similarity using Uparse software (Uparse v 7.0.1001). Representative sequence for each OTU was screened for further annotation using the Silva 132. Multiple sequence alignment was conducted using the MUSCLE software (Version 3.8.31). Alpha diversity (Chao 1, ACE, Shannon, Simpson, Observed-species, Good-coverage) was calculated with QIIME (Version 1.7.0), and beta diversity (weighed and unweighted UniFrac) was estimated with QIIME (Version 1.9.1). Both biodiversities were displayed with R software (Version 2.15.3).

RESULTS

HI Titers in the Birds Supplemented With Different Doses of YP

The changes in HI titers in chickens were depicted in Figure 1A, significantly higher HI titers were detected

Table 3. Experimental design (experiment 2).

Group	n	YP	Vaccination
A	15	0	
B	15	0	+
C	15	0.1%	+

Abbreviation: YP, yeast cell wall product.

in chickens supplemented with YP at 0.1% ($P < 0.05$) or 0.2% ($P < 0.01$) on 1 wk postimmunization, and in chickens supplemented with YP at 0.1% ($P < 0.01$) or 0.2% ($P < 0.05$) on 2 wk postimmunization than that in chickens only immunized with vaccine. Therefore, YP was dosed at 0.1% in the following experiment.

HI Titers in the Birds Supplemented With YP on Different Weeks

As shown in Figure 1B, HI titers were increased significantly in chickens supplemented with YP at 1 ($P < 0.01$), 2 ($P < 0.05$), and 3 ($P < 0.05$) wk postimmunization when compared with the chickens receiving NDV vaccine alone.

NDV-Specific IgA Level in Feces

The results were described in Figure 2. When compared with the chickens receiving NDV vaccine alone, significantly increased sIgA antibody in feces was recorded in chickens supplemented with YP at 2 ($P < 0.01$) and 3 ($P < 0.05$) wk postimmunization.

IgA + Cells

As shown in Figure 3A, the IgA + cells were round and stained yellow-brown. As shown in Figure 3B, when compared with the vaccine group, significantly increased IgA + cells were found in chickens supplemented with YP ($P < 0.05$) postimmunization.

Sequencing Data Analysis

The Illumina Nova was performed to generate a total of 1,124,449 raw sequence reads. The average of effective tags was 65,592 bp, and the average of Q30 was 97.89%. The detailed information for each sample was listed in Supplementary Material 1.

Microbial Correlation Among Groups

The YP exhibited a significantly higher observed species than the control ($P < 0.05$) (Figure 4A). It can be also discovered that there were significant differences in intestinal microflora richness index (Chao1) between the YP group and the control group ($P < 0.05$) (Figure 4B). Other alpha diversity index was shown in Supplementary Material 3.

The results of beta diversity analysis were depicted in Figure 5. It showed that the control and YP samples were separated well, and the principal components PC1 and PC2 explained 64.82 and 12.87% of the variation, separately.

Bacterial OTU Abundances in Phylum and Family Taxonomic Level

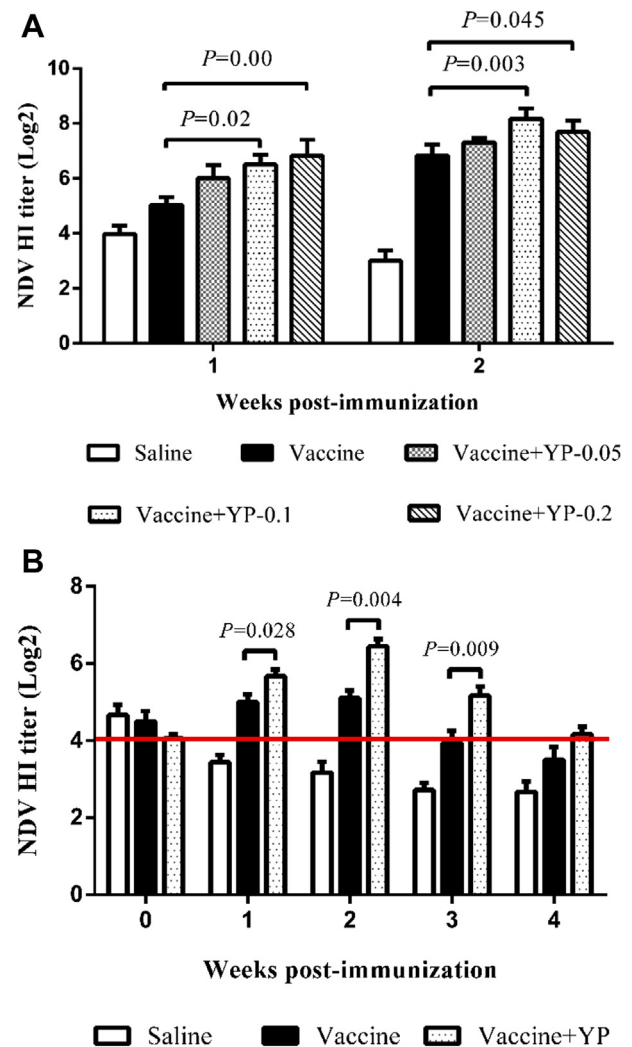Firmicutes, Bacteroidetes, Tenericutes, and Proteobacteria were found as the dominant microbiota on a

Figure 1. Antibody responses to vaccine of Newcastle disease virus (NDV) were assessed by HI titers. (A) Chickens (n = 15/group) were feeds with 0, 0.05, 0.1, or 0.2% YP and received oral immunization with a live NDV vaccine. Blood samples were collected 1, 2 wk postimmunization for analysis of NDV-specific HI titers. (B) Chickens (n = 15/group) were feeds with 0 or 0.1% YP and received oral immunization with a live NDV vaccine. Blood samples were collected before and 1, 2, 3, and 4 wk postimmunization for analysis of NDV-specific HI titers. The data were expressed as the log 2 of the highest dilution that exhibited complete inhibition of hemagglutination. The values were represented as mean ± SE. The red line represents the minimum positive antibody titer. Abbreviation: HI, hemagglutination inhibition.

phylum level (Figure 6A). Ruminococcaceae, Lachnospiraceae, and Bacteroidaceae were found to be the main microbiota at a family level (Figure 6B).

Effects of YP on Microbial Populations

The comparison of the top 5 cecum bacteria among groups in the phylum level was shown in Figure 7A. While no significant difference was found in the abundance of Actinobacteria between groups, significantly increased abundance of Firmicutes ($P < 0.05$) and Tenericutes ($P < 0.01$) and significantly decreased abundance of Bacteroidetes ($P < 0.01$), and Proteobacteria ($P < 0.05$) were observed in the YP group in comparison with the control group.
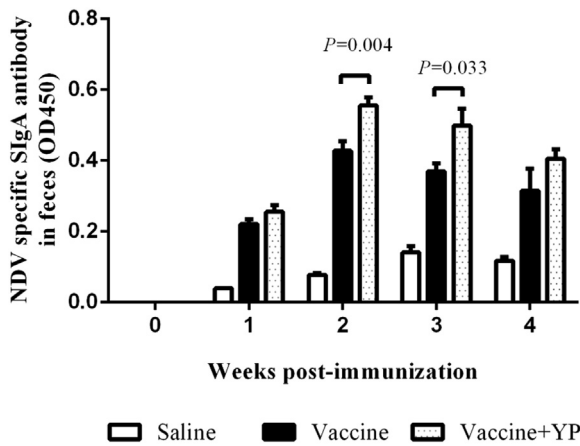
Figure 2. NDV-specific IgA in feces washing fluid was detected by ELISA. Chickens were fed with supplement of YP (0 or 0.1%) and received oral immunization with a live vaccine of NDV. Feces were collected before and 1, 2, 3, and 4 wk postimmunization for analysis of NDV-specific sIgA antibody. The values were represented as mean ± SE (n = 15). Abbreviations: NDV, Newcastle disease virus; YP, yeast cell wall product.

The comparison of the top 7 cecum bacteria among groups in the family level was shown in Figure 7B. Significantly higher abundance of Ruminococcaceae ($P < 0.05$) and significantly lower abundance of Bacteroidaceae ($P < 0.01$), Desulfovibrionaceae ($P < 0.05$), and Tannerellaceae ($P < 0.05$) were detected in the YP group than control.

DISCUSSION

The effects of dietary YP supplement on the intestinal IgA response and cecum microbiota after oral vaccination in chickens have been demonstrated in this study. The results showed that supplement of YP enhanced intestinal NDV-specific sIgA antibodies, IgA + cells, as well as serum NDV-specific HI titers in chickens after oral immunization of NDV vaccine. Moreover, YP supplementation significantly changed microbial community in cecum by decreasing abundance of Bacteroidaceae and increasing abundance of Ruminococcaceae.

Compared with the injection route, oral vaccination is much easier and can induce both IgG responses in serum and sIgA responses at gut mucosa for the prevention of infection. However, little is known about the effects of YP supplementation on the humoral immune responses and intestinal mucosal immune responses in chickens orally immunized with NDV vaccine. In the present study, significantly increased HI titers were found in chickens supplemented with 0.1 and 0.2% YP
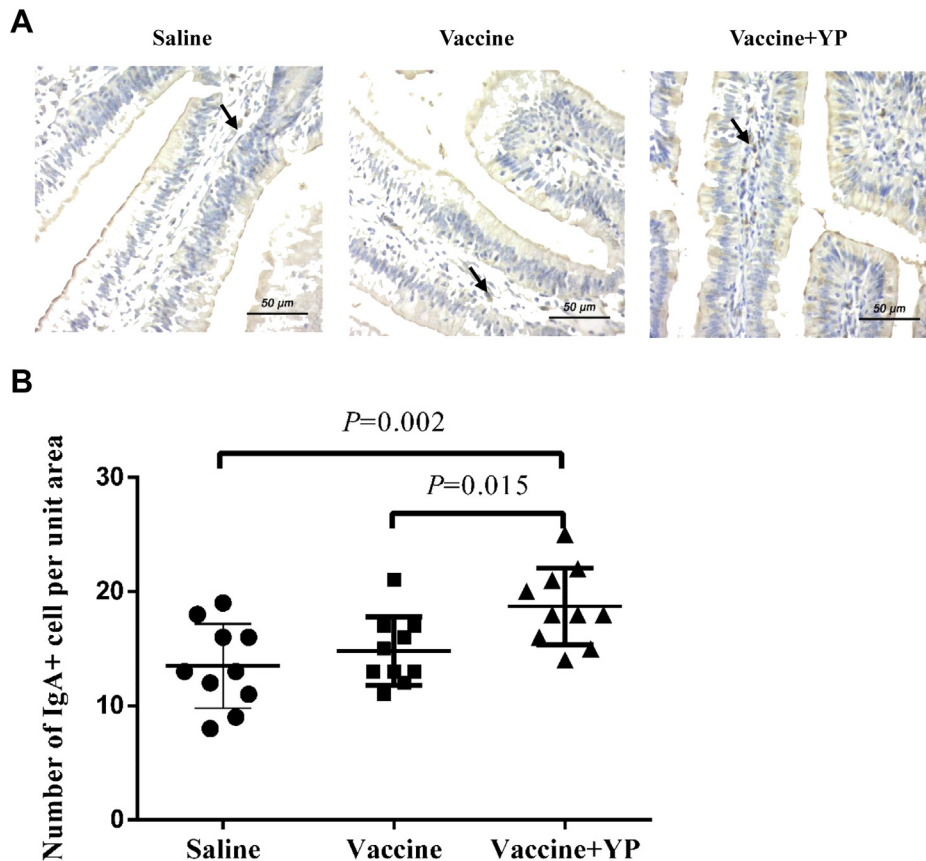

Figure 3. The number of IgA + cells. Chickens were fed with supplement of YP (0 or 0.1%) and received oral immunization with a live vaccine of NDV. The duodenum tissue samples were collected and fixed in 10% neutral buffered formalin (v/v) for immunohistochemical staining (400×). (A) IgA + cells of distributed in the lamina propria of the villi in the duodenum. Arrowheads represent positive cells. Scale bars: 50 μm. (B) Quantification of IgA + cell number at 10 different fields. The values were represented as mean ± SE (n = 10). Abbreviations: NDV, Newcastle disease virus; YP, yeast cell wall product.
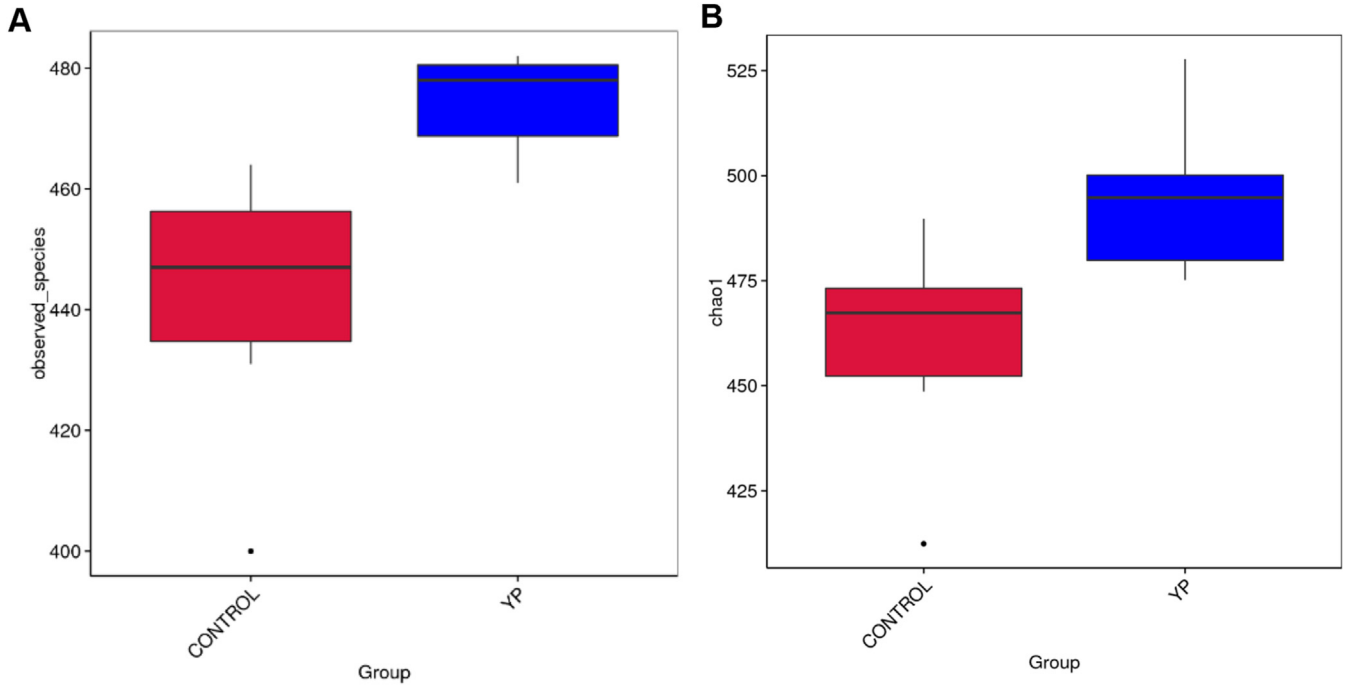
A

B

Figure 4. Alpha diversity between groups. (A) Average observed species and (B) average Chao1; CONTROL: normal feed and immunized with NDV vaccine, YP: normal feed with 0.1% YP and immunized with NDV vaccine. Abbreviations: NDV, Newcastle disease virus; YP, yeast cell wall product.
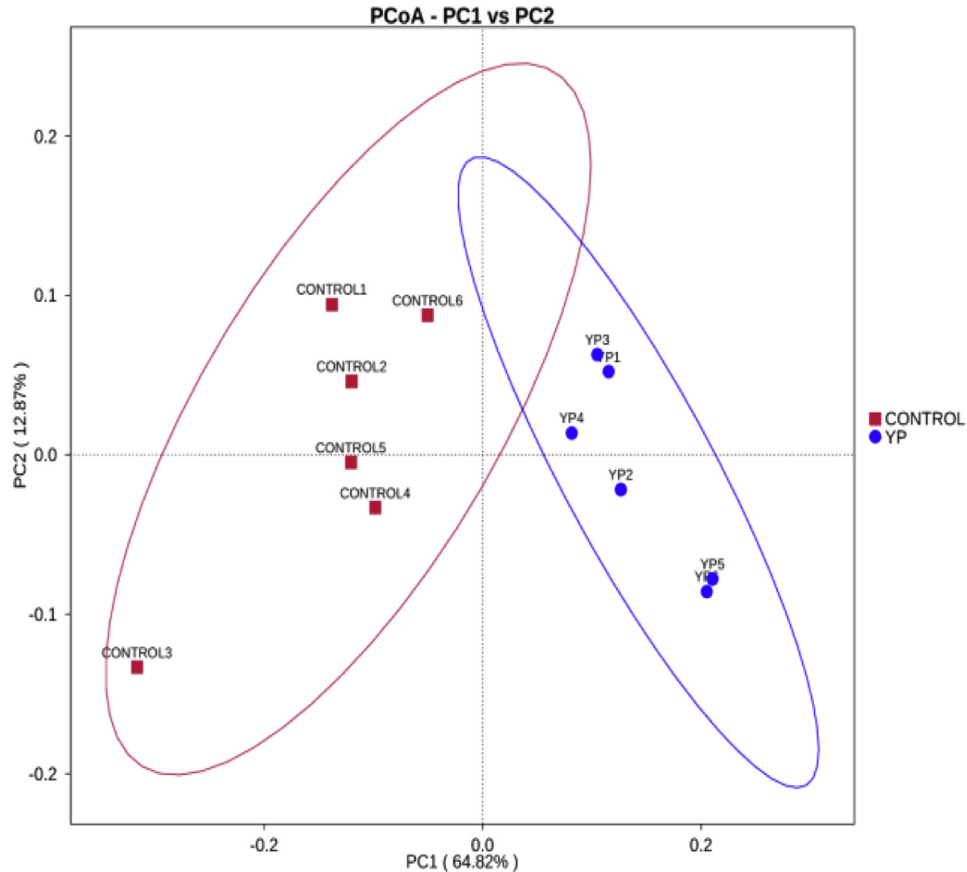

Figure 5. Beta diversity analysis between groups. Weighted PCoA plots of individual birds in each group. Individual sample was represented as spot with red (CONTROL: normal feed and immunized with NDV vaccine) and blue (YP; normal feed with 0.1% YP and immunized with NDV vaccine). Abbreviations: NDV, Newcastle disease virus; YP, yeast cell wall product.
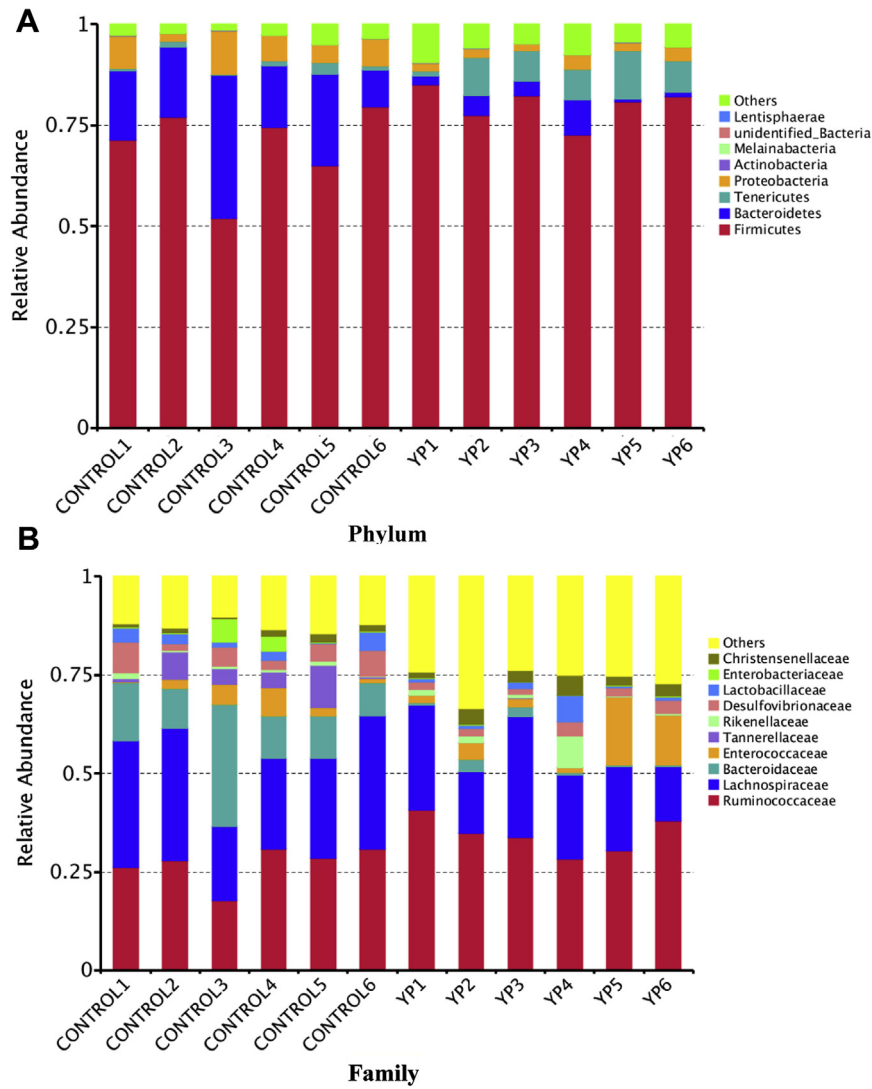
Figure 6. Classification of cecum flora compositions with (A) phylum level or (B) family level. CONTROL: normal feed and immunized with NDV vaccine, YP: normal feed with 0.1% YP and immunized with NDV vaccine. Abbreviations: NDV, Newcastle disease virus; YP, yeast cell wall product.
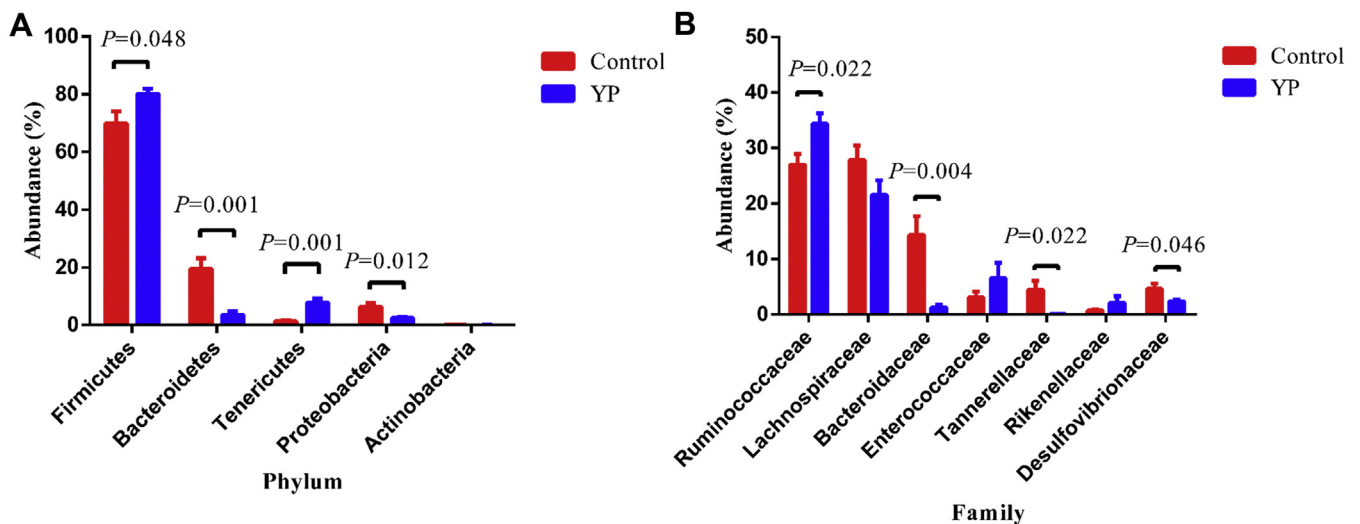

Figure 7. Comparison of cecum bacteria among groups in (A) phylum level or (B) family level. CONTROL: normal feed and immunized with NDV vaccine, YP: normal feed with 0.1% YP and immunized with NDV vaccine. The values were represented as mean ± SE (n = 6). Abbreviations: NDV, Newcastle disease virus; YP, yeast cell wall product.

(Figure 1A). Similar result has been observed in previous study. Muthusamy et al. (2011) have found that 0.1% yeast cell wall enhanced humoral immune response against NDV. The present study also showed that supplement of 0.1% YP increased NDV-specific HI titers which were maintained at high levels ($>4\log_2$) 3- and 4-wk postimmunization (Figure 1B), suggesting prolonged protection against ND virus in chicken peripheral blood. As YP was traditionally administrated via gastrointestinal route and could not be directly absorbed into peripheral blood, it is possible that YP displayed its immunomodulatory activity by triggering the intestinal mucosa immune system. Secretory IgA from B cells contributes to the maintenance of commensal/symbiotic bacteria in the gut, as well as protects mucosal surfaces against pathogens (Hodgkinson et al., 2017). Secretory IgA has multiple mechanisms for mucosal defense, including immune exclusion, prevention of microbial attachment, and neutralization of antigen (Corthesy, 2013; Mirhoseini et al., 2018; Turula and Wobus, 2018). In this study, higher NDV-specific sIgA of feces were investigated in the YP group than that in the control group (Figure 2). The enhanced specific sIgA may be attributed to MOS and β-glucan contained in YP, which were found to be effective in regulating intestinal mucosal immune competence in piglet and broiler chickens (Cox et al., 2010; Duan et al., 2019). Besides, increased IgA + cells in the laminal propria of intestine (Figures 3A, 3B) may also be an explanation for elevated sIgA production. Previous studies often detected the number of intestinal IgA + cells for evaluating gut mucosal immunity in mammals and birds. Xie et al. (2013) reported that polysaccharides extract from *Atractylodis macrocephalae koidz.* significantly increased intestinal IgA + cells in mice. Yu et al. (2015) observed that oral administration of ginseng stem and leaf saponins significantly enhanced the number of IgA + cells in duodenum of chicken.

The use of numerous yeast cell wall products in feeds could benefit chickens not only by enhancing productivity and immunity but also by regulating microbial flora populations. With the high-throughput next generation sequencing platforms rapidly developed, 16S rRNA gene amplicon sequencing has been widely utilized to identify the microbiota differences between control and treatments in poultry research (Mohd et al., 2015; Park et al., 2016). In this study, about 65,592 bp effective tags with high quality were generated (Supplementary Material 1). Park et al. (2016) reported that supplementation of yeast cell wall product was able to change cecum microbial composition in chickens. With the similar Illumina platform, we firstly investigated the effect of yeast cell wall product on gut microbiota in chickens after oral immunization. The microbiota of cecum played important roles in food digestion, water adsorption, as well as in host immune function (Berndt et al., 2007; Park et al., 2016; Huang et al., 2019). Although the relative abundance of microbiota in the cecum were distinctly abundant than that in small intestine, they shared similar dominant microbial populations such as Firmicutes, Proteobacteria, and Bacteroidetes in the phylum level (Sergeant et al., 2014; Mohd et al., 2015; Rychlik, 2020). In this study, Firmicutes, Bacteroidetes, Tenericutes, and Proteobacteria were found to be the dominant microbiota in cecum, which were consistent with previous reports (Figure 6B) (Park et al., 2017).

In the top 7 bacterial groups at the family level, abundance of Ruminococcaceae and Bacteroidaceae were found significantly different in YP and control (Figure 7B). Ruminococcaceae is profitable for the host as a keystone species inhabiting in the cecum and involved in the degradation of diverse fibers and polysaccharides (Fang et al., 2019; Mesnage et al., 2019). Interestingly, when compared with the control, YP treatment increased the abundance of Ruminococcaceae in the cecum. Similarly, Paes et al. (2020) reported significant higher abundance of Ruminococcaceae in rabbit fed with prebiotic supplementation contained MOS when compared with control. As MOS (a part component of YP) was degraded and fermented by Ruminococcaceae, we presumed that this substance then promoted its growth and predominance. Also, the increased population of Ruminococcaceae is correlated with improved intestinal permeability and declined inflammatory responses in other vertebrates, thus further studies are needed on whether YP could also have a protective effect on the intestinal barrier of chicken (Huang et al., 2015; Ren et al., 2019). The reduced population of the Bacteroidaceae at the family level (Figure 7B) and the *Bacteroides* and *Parabacteroides* at the genus level (Supplementary Material 2) suggested that YP might have inhibitory effects on growth of these microbial species. Although the exact reason for the decrease because of YP supplement is undefined, we speculated that it might be the MOS released from YP inhibits the utilization of glucose in the *Bacteroides* and *Parabacteroides* that resulting in declined population, as demonstrated in an in vitro model (Nakashimada et al., 2011).

It has been reported that manipulation of gut microbiome can influence adaptive immune response. Haghighi et al. (2005) observed a significantly increased antibody response to sheep red blood cells when treated with probiotics including *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in birds. Brisbin et al. (2011) found modulated systemic antibody- and cell-mediated immune responses in chickens by oral treatment with lactobacilli. In the present study, enhanced NDV-specific HI titers and sIgA were associated with modulated cecum bacteria such as Ruminococcaceae and Bacteroidaceae. Though the exact mechanism that how bacteria influence adaptive immune response remains to be elucidated, it is speculated these bacteria might modulate the immune response through stimulating the production of cytokines (Oakley and Kogut, 2016).

Based on the results of enhanced serum HI titers, intestinal sIgA response, and the beneficial effect on gut microbiota, YP deserves further study as an alternative for improving oral vaccination and intestinal ecosystem in chickens.

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DISCLOSURES

The authors declare no competing financial interests.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.psj.2020.09.075.

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